KINETIC STUDY ON POLY(3-HYDROXYBUTYRATE) PRODUCTION FROM OIL PALM FROND JUICE IN BATCH FERMENTATION

NURUL AMALINA BINTI ALIAS

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2014

©NURUL AMALINA BINTI ALIAS (2014)

ABSTRACT

This research is about the kinetic study on poly(3-hydroxybutyrate), P(3HB) production from oil palm frond juice in batch fermentation. Batch fermentation experiment was conducted in 500 mL shake flasks by using *Cupriavidus necator* in order to produce P(3HB). Fresh oil palm frond (OPF) was pressed and undergoes certain processes such as filtration, centrifugation and sterilization to get oil palm frond juice. OPF juice were used as the fermentation substrate for P(3HB) production. In this study, the microbial growth (μ, μ_{max}, $Y_{X/S}$, K_s) and product ($Y_{P/S}$, $Y_{P/X}$) kinetic parameter can be obtained by using MATLAB R2009b software. The models were fit to the data using Runge-Kutta 4^{th} order Method by minimizing the error between experimental and predicted data using Simplex Method. Logistic Model was developed for the biomass growth model. Model for polyhydroxyalkanoates (PHA) was developed based on assumptions that each cell contains the same amount of PHA and it degrades at the same rate. Predicted data was obtained using function ode45 in MATLAB R2009b software which implements Runge-Kutta 4^{th} Order Method. Based on the results obtained, it shows that the predicted data of biomass profile was fitted well to the experimental data but for PHB profile, the predicted data deviated quite far from the experimental data. This shown that the models developed were representing biomass growth very well. Kinetic parameters for microbial growth and production of P(3HB) were determined by using Monod equation. The values are μ_{max} = 0.0346h^{-1}, Ks = 0.0930g/L, μ = 0.0344h^{-1}, Yx/s= 0.4731 and $Y_{P/S}$ = 0.0416.

ABSTRAK

Kajian ini adalah berkenaan kajian kinetik bagi menghasilkan poly(3-hydroxybutyrate) daripada jus pelepah kelapa sawit menggunakan proses penapaian. Eksperimen prosess penapaian dijalankan di dalam 500 mL kelalang goncang menggunakan *Cupriavidus necator* untuk menghasilkan P(3HB). Pelepah kelapa sawit yang segar diperah dan melalui beberapa proses seperti penapisan, pengemparan dan pensterilan untuk mendapatkan jus pelepah kelapa sawit. Jus pelepah kelapa sawit digunakan sebagai pemangkin di dalam proses penapaian untuk menghasilkan P(3HB). Dalam kajian ini, pertumbuhan mikrob (μ, μ_{max}, $Y_{X/S}$, K_s) dan produk ($Y_{P/S}$, $Y_{P/X}$) parameter kinetik boleh didapati menggunakan perisian MATLAB R2009b. Data ramalan diperolehi dengan mengaplikasikan Kaedah Numerikal Runge-Kutta Peringkat Ke-4 disamping meminimakan ralat antara data eksperimen dan data simulasi menggunakan Kaedah Simplex. Model logistik telah dibangunkan untuk model pertumbuhan biojisim. Model polyhydroxyalkanoates (PHA) telah dihasilkan berasaskan andaian bahawa setiap sel mengandungi jumlah PHA yang sama dan ia mengurang pada kadar yang sama. Data jangkaan telah diperolehi menggunakan fungsi ode45 dalam perisian MATLAB R2009b yang melaksanakan Runge-Kutta Peringkat Ke-4. Berdasarkan hasil eksperimen yang diperolehi, ia menunjukkan bahawa data ramalan profil biomass telah dihasilkan dengan baik kepada data eksperimen tetapi untuk profil PHB, data ramalan menyimpang jauh dari data eksperimen. Ini menunjukkan bahawa model yang dibangunkan telah mewakili pertumbuhan biomass dengan baik. Parameter kinetik untuk pertumbuhan mikrob dan pengeluaran P(3HB) ditentukan dengan menggunakan persamaan Monod. Nilai yang diperolehi adalah μ_{max} = 0.0346h^{-1}, K_S = 0.0930g/L, μ = 0.0344h^{-1}, $Y_{X/S}$ = 0,4731 dan $Y_{P/S}$ = 0,0416.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

PHA	Polyhydroxyalkanoate
P(3HB)	Poly (3-hydroxybutyrate)
OPF	Oil palm frond
OPFJ	Oil palm frond juice
$(NH_4)_2SO_4$	Sodium hydrogen sulphate
HPLC	High performance liquid chromatography
H_2SO_4	Sulphuric acid
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Potassium hydrogen phosphate
$MgSO_4$	Magnesium sulphate
NaOH	Sodium hydroxide
rpm	Rotation per minute
μ	Growth rate constant
μ_{max}	Maximum growth constant
S	Substrate concentration
K_M	Half saturation coefficient
g/L	Gram per liter
y_1	Biomass concentration
y_2	PHB concentration
t	Time
k_1	Parameter represent the growth rate
k_2	Final biomass concentration
k_3	Proportionality parameter
k_4	Loss rate of PHB

1 INTRODUCTION

1.1 Background of study

Recently, bio-based and biodegradable products have raised great interest to bring a significant contribution to the sustainable development. Concerns about these environmental friendly materials were increasing as they contribute lot of advantages. In medical field, polymers have a growing importance as niche products for special applications (Koller et al., 2008). Other than that, there is a rapidly increasing need for polymeric compounds acting as packaging materials. Biodegradable products can reduce problems like greenhouse effect and global warming (Koller et al., 2008). Furthermore, there are more waste of highly resistant plastics that are not incinerated is piled every year. Recycling systems need a certain degree of purity of the plastics to be reutilized and a sorting accuracy so that they can function sufficiently to solve the problems. Moreover, the costs for collection are quite high and recycling also will give a negative impact such as increase in brittleness for the mechanical properties of the materials (Braunegg et al., 2007). It becomes an environmental necessity to stop the negative development by switching to alternative strategies instead of using fossil resources and support the bio-based materials.

Polyhydroxyalkanoates (PHAs) can be categorized as polyesters (Koller et al., 2008). It can be produced classically by numerous prokaryotic strains from renewable resources like carbohydrates, lipids, alcohols or organic acids (Koller et al., 2008). Other than that, the production must be under unfavourable growth conditions due to imbalanced nutrient supply (Zahari et al., 2012). Polyhydroxyalkanoate can be divided into two types which are short chain length PHAs and medium chain length PHAs (Annuar et al., 2007). Annuar et al., 2007 also stated that poly (3-hydroxybutyrate) is one of the typical examples of short chain length polyhydroxyalkanoates and it can be said as biodegradable thermoplastic polyester. It can be produced by using microorganisms such as *Cupriavidus necator*.

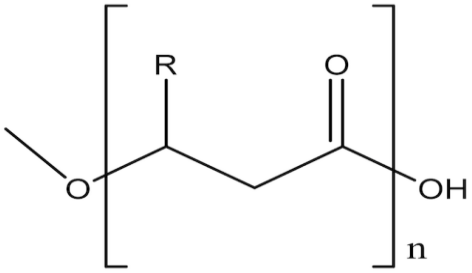

Figure 1-1: General structure of polyhydroxyalkanoates (PHAs) (Green, 2010)

It has been reported that *Cupriavidus necator* is well known as polyhydroxyalkanoates (PHAs) producer and its ability to accumulate PHAs more than 50 wt. % (Zahari et al., 2012). Based on the report also, it was stated that oil palm frond (OPF) juice was used as the novel and renewable feedstock to produce poly (3-hydroxybutyrate), P(3HB). It was reported that OPF juice is a good substrate for P(3HB) production by using the wild-type of *Cupriavidus necator*. The juice can be obtained simply by pressing the fresh OPF (Zahari et al., 2012).

Oil palm frond juice contain sugars mainly glucose as well as nutrients and minerals that essential for bacterial growth in order to produce poly (3-hydroxybutyrate) (Zahari et al., 2012). Moreover, the use of oil palm frond juice is advantageous since the processes to obtain the sugars are simple and no harsh pre-treatment steps needed compared to other lignocellulosic materials. In addition to that, OPF is an oil palm biomass which is produced as by-products of the palm oil industry. Zahari et al., 2012 also stated that utilization of OPF as an alternative and renewable source of raw material for the production of P(3HB) can reduce the dependence on food crops such as sugar cane, tapioca starch, corn starch, soybean, and etc.

It always been somewhat challenging in modelling the biological processes (Kiviharju et al., 2006). Ideal mixing is the easiest modelling concepts presume, which is rarely, if ever, achieved. On top of that, the asepticity requirements make measurements from the reactor non-trivial. Cellular processes, mass transfer and control aspects make the modelling task appear even more daunting, and thus it should carefully be considered, what really needs to be modelled given the specific problem. Besides, different tools have been developed in recent years for different modelling needs (Kiviharju et al., 2006).

1.2 Problem statement

Recently, there is not much research about the kinetic parameter of the production of poly (3-hydroxybutyrate) such as the yield, the maximum specific growth rate and many more, so it is important to determine the parameters. There are no formal kinetic model for the microbial growth and the production of poly (3-hydroxybutyrate). On top of that, lots of researches have been conducted to discuss and propose methods to reduce the cost since the production cost of poly (3-hydrobutyrate) are quite high. Sustainable and renewable substrates like oil palm frond juice have been utilized as it becomes an important objective for bioplastic commercialization. In this study, the microbial growth and product kinetic parameter for the production of poly (3-hydroxybutyrate) were determined by mathematical models.

1.3 Objective of the research

This research is conducted to determine the kinetic parameter for poly(3-hydroxybutyrate), P(3HB) production from oil palm frond (OPF) juice.

1.4 Scopes of study

The scopes of study used in this research were:

I. The kinetic parameter for the production of poly(3-hydroxybutyrate), P(3HB) are including yield, maximum specific growth rate, substrate constant and many more.
II. Oil palm frond juice will be used as the sole carbon source in this research in order to produce poly (3-hydroxybutyrate) because it is renewable and cheap carbon source compared to the commercial substrates.
III. Batch fermentation process was conducted in shake flask experiment. Batch fermentation was chosen in this research because of several reasons which are the duration process of chemical reactions are fixed and the processing times are assumed constant. In addition, the process is uninterrupted since the fermenter is filled once until its full and no more substrates added through reaction.

2 LITERATURE REVIEW

2.1 Introduction

This chapter contains the description of study development based on the literature. The subtopics of this chapter include fermentation of polyhydroxyalkanoates, the need for cheap substrates, determining cell mass concentration, kinetic models, MATLAB R2009b software and Runge-Kutta 4th order method.

2.2 Fermentation of PHA

Polyhydroxyalkanoates (PHAs) can be classed as linear polyester compounds that can be naturally produced by many bacterial organisms (Green, 2010). PHAs can be produced from renewable resources and biodegraded easily. Moreover, PHAs have similar structural properties of many conventional plastics. Nowadays, there is currently great interest in using PHAs as alternative to petroleum-derived plastics because PHAs are ecologically safe polymers. By using PHAs, harmful toxic compounds can be avoided.

As described by Makka and Casida (1987), *Cupriavidus necator* was used to accommodate a non-obligate bacterial predator of various gram-negative and gram-positive soil bacteria and fungi. *Cupriavidus necator* which was formerly known as *Ralstonia eutropha* is a versatile organisms that will be used for batch fermentation process in the production of PHAs and at present, bacterial fermentation of *Cupriavidus necator* is used widely in industrial processes towards PHAs (Khanna and Srivasta, 2005). *Cupriavidus necator* can accumulate PHAs up to 80% (wt.) of dry cell mass using various carbon sources including carbohydrates, alcohols and organic acids (Lee and Gilmore, 2005).

2.3 The need for cheap substrates and its occurence

Application of biotechnological processes for industrial production can be regarded as promising for sustainable development even the range of products biotechnological production strategies have not yet passed the economic viability test (Koller et al., 2010). Cost of materials can be said as one of the reasons. On top of that, the utilization of a broad range of waste that can be upgraded to play the role of feed-stocks for the

bio-mediated production of desired products can be identified (Koller et al., 2010). There are lots of biotechnological products produced are closely related to agriculture (Khardenavis et al., 2007).

Production of PHAs especially the economics is determined to a great extent by the cost of the raw materials up to 50% of the entire production costs (Koller et al., 2010). PHAs can accumulate under aerobic conditions, thus resulting high losses of the carbon substrate by intracellular respiration. Maximum amount of less than 50% of the carbon source are for PHA production and directed towards biomass (Koller et al., 2010). Waste material like oil palm frond playing an important role as it constitutes a good strategy for cost-efficient in the biopolymer production.

2.4 Determining cell mass concentration

Direct methods and indirect methods can be used to determined cell mass concentration (Shuler and Kargi, 2002). Cellular dry weight determination is the most commonly used direct method in order to determine cell mass concentration. This method is applicable for cells grown in solids-free medium. On the other hand, the dry weight measurement will be inaccurate if the non-cellular solids such as molasses solids, corn steep liquor or cellulose are present. In this method, the samples are centrifuged or filtered and washed with a buffer solution or water. After that, the washed wet cell mass is then dried for at least 24 hours and the cell weight is measured.

There is another method that can be used based on absorption of light by suspended cells in sample culture media (Shuler and Kargi, 2002). Spectrometer can be used to measure the intensity of the transmitted light. Besides that, in the absence of other solids or light absorbing compounds, optical density or turbidity measurement of the culture medium provides a simple and inexpensive method of estimating cell density. The amount of transmitted light decreases and the absorbance reading on spectrometer increases as the bacterial cell population increases.

2.5 Kinetic models for modelling

Behaviour of microorganisms under different physical or chemical conditions can be described by using kinetic model (Ismail and Salihon, 2010). Microbial safety or shelf life of products can be predicted by using the models. On top of that, the critical parts of the production and distribution process can be detected. Kinetic models can be built by

measuring and modelling the growth. Application of an unstructured kinetic model was the easiest kinetic modelling technique. Besides, the kinetics can be linear or non-linear, single-phase or multiphase. Monod model has been defined as the most popular kinetic model (Kiviharju et al., 2006). It contains two parameters that define the relation of growth and substrate consumption.

$$\mu = \mu_{max} \frac{S}{S + K_s}$$

Where

μ = growth rate constant

μ_{max} = maximum growth rate constant

S = substrate concentration

K_s = half saturation coefficient

However, the Monod equation empirically fits a wide range of data satisfactorily and is the most commonly applied unstructured, non-segregated model of microbial growth (Shuler and Kargi, 2002). Substrate- limited growth was described by Monod equation only when growth is slow and population density was slow. In addition, environmental conditions can be related simply to S under these circumstances. If the consumption of a carbon-energy substrate is rapid, the release of toxic waste products is more likely (due to energy-spilling reactions). Besides, at high population levels, the build up of toxic metabolic by-products becomes more important.

2.6 Advantages of kinetic models

In order to build kinetic models, the rules are well rooted in the theories on thermodynamics and chemical kinetics (Boekel and Tijskens, 2001). Fundamental and generic models can be obtained by consistent application of these rules. Moreover, with generic models, extrapolations in areas outside the testing area are allowed, provided the processes are governed by the same mechanisms. It means that, the model parameters can be validated on separate data sets. The separate data sets can be obtained in favourable laboratory circumstances for example and can be applied in practical situations. In addition, the transfer of parameter values is possible without any difficulty. Boekel and Tijskens, 2001 also stated that scaling-up problems can be

avoided or diminished if the kinetic modelling were consistent applied. For fundamental processes and batch parameters, a distinction can be made. Furthermore, kinetic parameters are specific for a certain process and they have the same value for each duplication or repetition. Batch parameters will depend on the composition of actual batches of agricultural products.

2.7 Matlab R2009b software

MATLAB is the flagship software product of The MathWorks, Inc. (Chapra et al., 2010). MATLAB is originally developed as a *matrix* laboratory. Other than that, mathematical manipulations of matrices are very conveniently implemented in an easy-to-use, interactive environment. MATLAB has added a variety of numerical functions, symbolic computations and visualization tools to these matrix manipulations. Furthermore, MATLAB has a variety of functions and operators that allow convenient implementations of many of the numerical methods. Some of important features in MATLAB differentiate it from other high-level languages (Recktenwald, 2000). The programs in MATLAB are interpreted, not compiled. There are two different kinds of MATLAB programs which are scripts and functions. In addition, programs can be written as so called M-*files* that can be used to implement numerical calculations (Chapra et al., 2010).

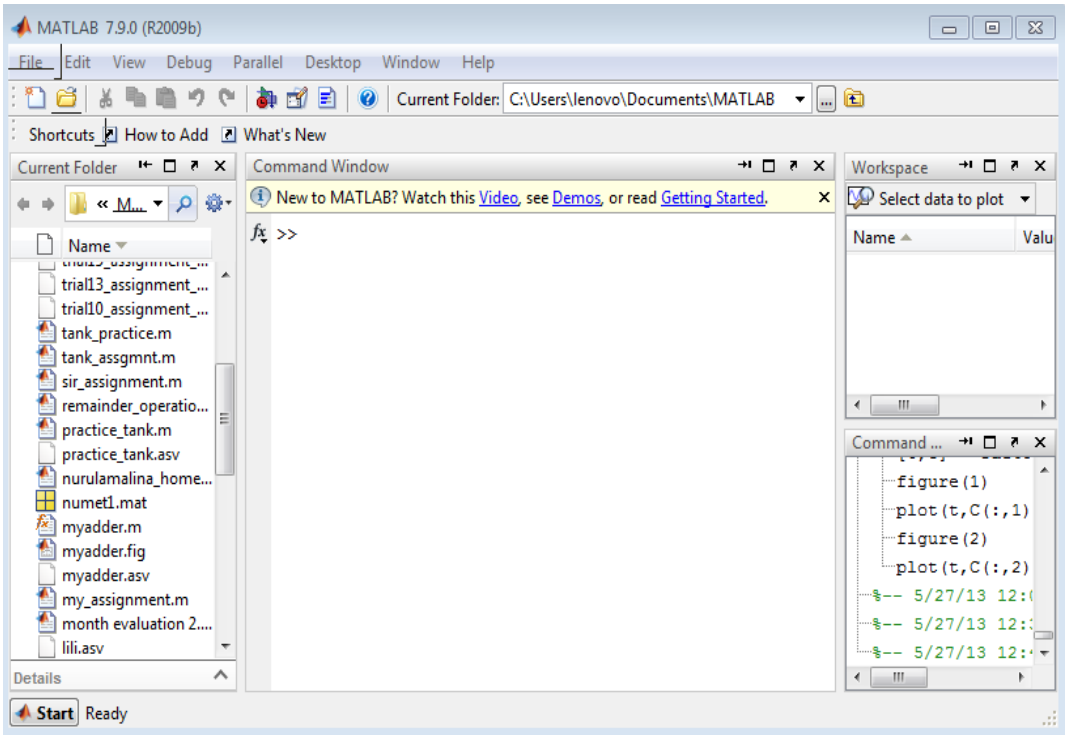

Figure 2-1: Command window in MATLAB R2009 software

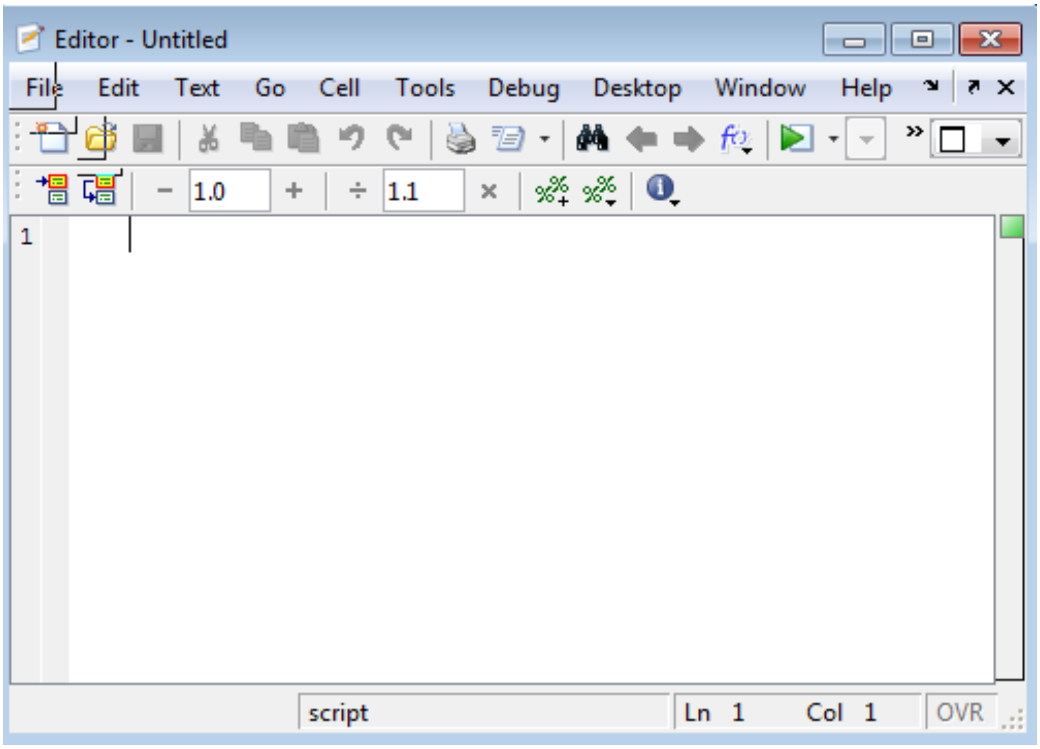

Figure 2-2: M-files window in MATLAB R2009b software

2.8 Fourth-order Runge-Kutta methods

Recently, the most popular Runge-Kutta methods are 4th order (Chapra et al., 2010). There are an infinite number of versions as with the 2nd order approaches. The following equation is the most commonly used form, and it was called classical fourth order Runge-Kutta method.

$$x_{i+1} = x + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4)\, h$$

where

$$k_1 = f(t_o , x_o)$$

$$k_2 = f\left(t_o + \frac{1}{2}h, x_o + \frac{1}{2}k_1 h\right)$$

$$k_3 = f\left(t_o + \frac{1}{2}h, x_o + \frac{1}{2}k_2 h\right)$$

$$k_4 = f(t_o + h, x_o + k_3 h)$$

The function of ode45 was used in this study and has been developed in MATLAB based on this algorithm which is also known as Runge-Kutta 4th order method. Besides, in this algorithm, the integration interval from 0 to the global residence time (t) was divided into N

sub-intervals with h = t/N. Dupal et al., 2007 stated that Runge-Kutta 4_{th} order method is the best solution for ODE and this method is still the highest consistent accuracy with low error and it is efficient for any condition more than other methods.

3 MATERIALS AND METHODS

3.1 Microorganisms

Cupriavidus necator (NCIMB11599) obtained from the Culture Collection, University of Goteborg, Sweden which is also known as *Ralstonia eutropha* has been used widely by using several carbon sources for the production of P(3HB).

3.2 Cultivation of bacteria

According to Zahari et al. (2012), 5.0 g of peptone, 3.0 g of yeast extract, 10 g of glucose and 15 g agar powder were mixed in a 1.0 L of distilled water by using Scott bottle as the sterile nutrient rich medium to prepare agar plate. The solution will be stirred using magnetic stirrer and autoclaved at 121^{o}C for 30 minutes. Scott bottle was wrapped with aluminium foil loosens to avoid build up of pressure in the bottle during autoclave. When it reached 50^{o}C after autoclave process finished, the agar must be cool down. 10ml of agar solution will be transferred into a petri plate and let the solution solidify for 15 minutes. The agar solution was transferred into 20 petri plates and the remaining agar solutions keep in refrigerator. After the agar solution solidified, the bacteria from culture need to be transferred onto the agar plate by using inoculating loop in the laminar air flow hood with bunsen burner lighted on for sterilization. The agar plates contain the bacteria were incubated in incubator for 24 hours at 30^{o}C. After 24 hours, the agar plates will be transferred into -4^{o}C refrigerator to avoid contamination.

3.3 Preparation of oil palm frond juice

The oil palm frond juice will be extracted by pressing the frond using sugar cane press machine. The oil palm frond juice was centrifuged at 15,000 rpm for 15 min at 4^{o}C and the supernatant was filtered using a mixed cellulose eater membrane ester with pore size between 3 and 5 µm. Before use, the filtrate was stored at -20^{o}C. To study amount of sugar, composition and distribution in the OPF juice, several branches of fresh oil palm frond without leaves was cut into 1.0 m length at three different section, Initial(X), Middle (Y), and Edge (Z) as explained by Zahari *et al*., 2012.

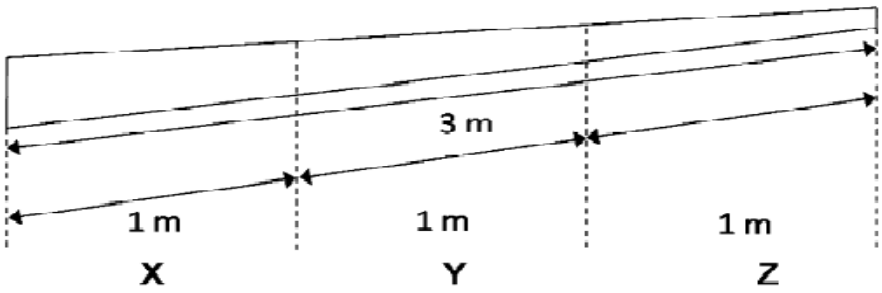

Figure 3-1: Schematic diagram of fresh oil palm frond (OPF) without leaves (X, Y, and Z representing three different section).

Source: (Zahari *et al*., 2012)

3.4 Fermentation process

20 ml of mineral salt medium (6.7 g of KH_2PO_4, 1.5 g of K_2HPO_4, 1.0 g of $(NH_4)_2SO_4$ and 0.2 g $MgSO_4$ mixed in a 1L Scott bottle) were prepared into sterilized 500 ml Erlenmeyer shake flask and ultra pure water was added. The pH need to be adjusted accordingly with 2 M NaOH. The mineral salt prepared must be autoclaved at 121^{o}C for 20 minutes. The flask opening must be covered with sterile cotton and shaken slowly. The Erlenmeyer flask has to be incubated for 48 hours and for every 6 hours, sample were taken in order to get the profile data.

Figure 3-2: Samples that have to be taken for every 6 hours

3.5 Cell dry weight analysis

In order to determine the amount of cell concentration in the cell, cell dry weight analysis can be used. An empty eppendorf tube must be dried for 48 hours in oven. Then, 1 ml of the sample is put into the dried eppendorf tube and centrifuged at 10000 rpm for 5 minutes by using biofuge. Ultra pure water will be added into the eppendorf tube after remove the supernatant. Centrifuged the tube again at 10000 rpm for 5 minutes and remove the supernatant. The remaining pellet will be dried in the oven for 48 hours at 60^{o}C and the weight of the tube with the pellet will be recorded. The sample cell dry weight divide with the volume will be taken to determine the biomass concentration.

Figure 3-3: Biofuge

3.6 Analysis of P(3HB)

P(3HB) analysis must be done in order to determine P(3HB) content. 1ml of the product sample is put into eppendorf tube and centrifuge at 10000 rpm for 5 minutes by using biofuge while the pellet must be dried in oven for 24 hours, remaining supernatant must be removed. Then, 1 ml of concentrated H_2SO_4 was added to dried pellet to digest it. After that, the eppendorf tube has to be closed and put in the oven at 90^{o}C at 2 hours. The pellet has to be transferred into a test tube and 9 ml of ultra pure water was added into the test tube after 2 hours. The test tube content must be mixed well by using vortex. Lastly, 1 ml sample from the test tube was filtered using 0.45 µm microfilter using 1 ml needle syringe. The filtered sample was transferred into High Performance Liquid Chromatography (HPLC) brand Agilent Technologies from Faculty of Chemical Engineering & Natural Resources labaratory vial. The sample was analysed and the concentration of P(3HB) was calculated based on HPLC results.

Figure 3-4: High Performance Liquid Chromatography (HPLC)

3.7 Models development

Biomass *Cupriavidus necator* growth can be represents by using logistic model. Since we lack of understanding on biosynthesis of polyhydroxyalkanoates (PHA), empirical models were used. Moreover, the empirical model represented PHA production have to be developed based on assumptions that each cell contain the same amount of PHA and that PHA degrades with the same rate. Logistic model was one of the best models for simulations of growth and glucose consumption in batch growth phase (Kiviharju et al., 2006).

3.8 Fitting methods

As a step to fit models for experimental data, Matlab R2009b will be used. Some functions are required in Matlab in order to solve the models that will be developed. These functions can be developed by using M-*file*. Once the functions have been developed, we can obtain the value of parameters needed for the models with minimum error between predicted data and experimental data calculated (Ismail and Salihon, 2010). All of these functions algorithms integrated Runge-Kutta 4_{th} Order Method and Simplex Method with the models developed. Two functions in M-*files* were developed in order to determine four parameters which are k_1, k_2, k_3 and k_4. Furthermore, the first M-*file* was aimed to obtain values for parameters k_1 and k_2 for the first model developed. This first model developed was represents biomass growth with notation as y_1. Then, the second M-*file* was aimed to obtained values for parameter k_3 and k_4 for the second model developed. Production of desired product which is PHA in this study was represented by the second model developed. In addition, both M-*files* were used

fminsearch function to minimize the error between predicted data and experimental data. Ismail and Salihon, 2010 also mentioned that the fminsearch function was calculated error and suggested new values of parameters until minimum error were reached. The values of parameters k_i obtained as the output of this function. Once the values of parameters were obtained, these values were inserted into third M-*file* to generate predicted data and each M-file has different calling command typed in command window.

3.9 Models performance test on data

After models development and fitting methods steps, the function systems were tested on a set of experimental data of biomass *Cupriavidus necator* growth.

3.10 Generate data

The same models and fitting methods were used on data of biomass *Cupriavidus necator* growth and production of intracellular biopolymer P(3HB) once the model performance test shows a positive results.

4 RESULTS AND DISCUSSIONS

4.1 Introduction

In this chapter, it contains results and discussions for this research. The results were discussed more under each subtopics of this chapter.

4.2 Development of models

Four assumptions were made in order to develop Logistic Model. The first and second assumption stated that *Cupriavidus necator* cell at any particular point in time contains the same amount of PHB and PHB level determined represents the nett amount resulting from synthesis and loss since PHB may become carbon sources for the cells in substrate limitation condition. These assumptions lead to,

$$\frac{dy_1}{dt} = k_1 y_1 - \frac{k_1 {y_1}^2}{k_2}$$

Where:

y_1 is biomass concentration (g/L)
k_1 is parameter represent the growth rate (hr-1)
k_2 is final biomass concentration (g/L)
t is time (hr)

Third assumption was made stated that *Cupriavidus necator* are equally capable of at any particular point in time and PHB are producing at the same rate. The rate of production of PHB in the fermentation at any particular point is proportional to the amount of *Cupriavidus necator* present. The third assumption gives,

$$\frac{dy_2}{dt} = k_3 y_1$$

Where:

y_2 is PHA concentration (g/L)
k_3 is proportionality parameter.

The fourth assumption stated that the loss of PHA takes place in each of the *Cupriavidus necator* at the same rate and for the last assumption we made stated that if the PHA was not being formed at the same time as it was being lost, and that in the whole *Cupriavidus necator* cells the same mechanism of destruction was in operation.

The fourth assumption gives,

$$\frac{dy_2}{dt} = k_3 y_1 - k_4 y_2$$

Where k_4 is loss rate of PHA (hr-1)

4.3 Models performance on data

Data for biomass *Cupriavidus necator* growth and production of PHB were generated. As for the results, the values of ki obtained were shown in Table below. The comparison graphs for biomass and PHB production were shown in the Figure 8 and 9 respectively. From the graphs plotted, we can see that the curve predicted data for biomass approached each point of experimental data for particular point in time while the curve predicted data for PHB production does not approached the point of experimental data. It deviated may be because the values of ki were not converged.

Table 4-1: Values of parameters k_i

k_1	k_2	k_3	k_4
0.0453	20.3423	0.2010	2.8030

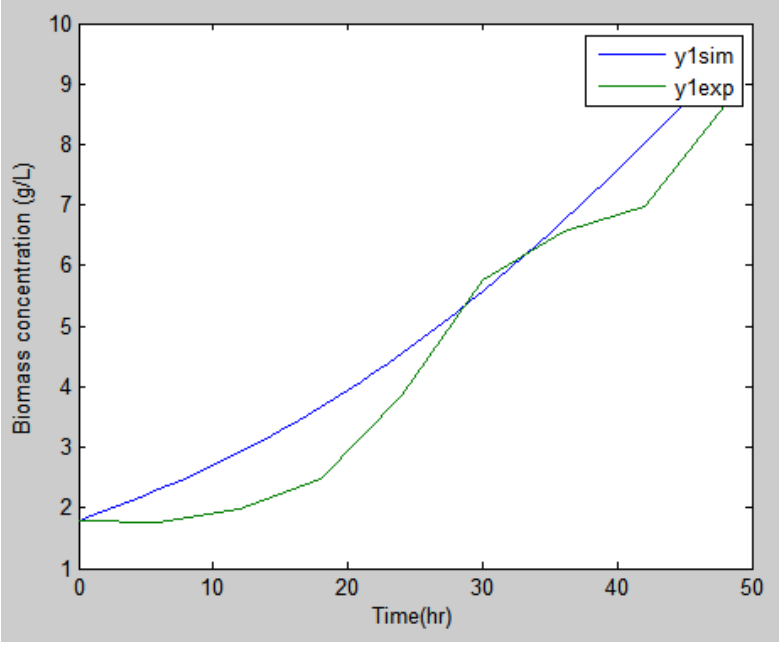

Figure 4-1: Graph of biomass concentration (g/L) versus time (hr)

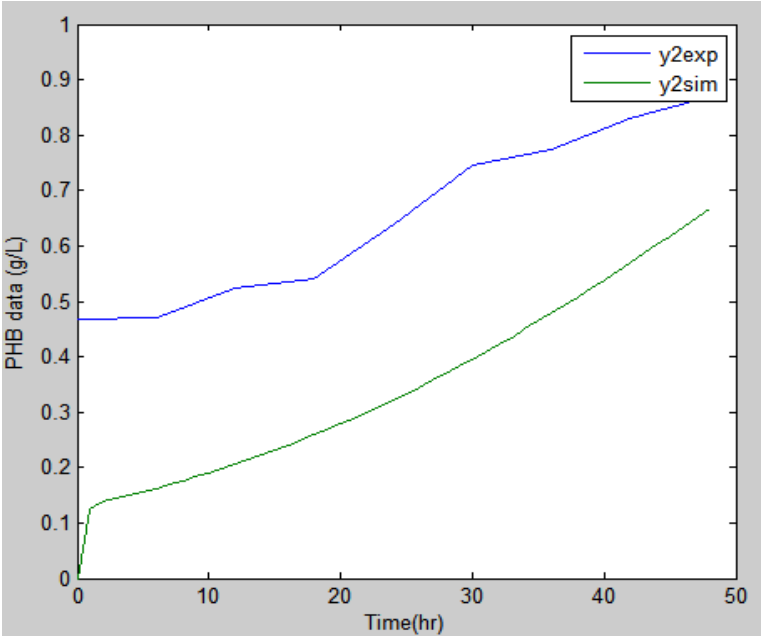

Figure 4-2: Graph of biomass concentration (g/L) versus time (hr)

4.4 Values of kinetic parameters

Kinetic parameters for microbial growth and production of P(3HB) were determined by using Monod equation (Chapra,2010).

$$\mu = \mu_{max} \frac{S}{S + K_s}$$

Where

μ = growth rate constant

μ_{max} = maximum growth rate constant

S = substrate concentration

K_s = half saturation coefficient

Table 4-2: Values of kinetic parameters

Kinetic parameters				
μ_{max} $= 0.0346\ h^{-1}$	K_S = 0.0930 g/L	μ $= 0.0344\ h^{-1}$	$Y_{\frac{X}{S}}$ = 0.4731	$Y_{\frac{P}{S}}$ = 0.0416